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     (FILE 'HOME' ENTERED AT 11:53:51 ON 21 JUN 2002)

     FILE 'BIOSIS, CAPLUS, EMBASE, SCISEARCH, CANCERLIT, BIOTECHNO, MEDLINE'
     ENTERED AT 11:54:26 ON 21 JUN 2002
L1        74467 S (SIALIC ACID) OR (NANA)
L2        81959 S (SIALIC ACID) OR (NANA) OR (N-ACETYLNEURAMINIC ACID)
L3          415 S L2 (S) PYRUV?
L4          510 S L3 (S) (N-ACETYLMANNOSAMINE) OR (MANNAC)
L5          317 S L4 AND (SYNTHE? OR BIOSYNTHE?)
L6           89 DUP REM L5 (228 DUPLICATES REMOVED)
L7           15 S L6 AND ALDOLASE
L8            8 S L6 AND SYNTHETASE
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L8 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:413417 BIOSIS
DOCUMENT NUMBER: PREV199799705460
TITLE: Purification and characterization of the Escherichia coli K1 neuB gene product N-acetylneuraminic acid **synthetase**.
AUTHOR(S): Vann, Willie F. (1); Tavarez, Jose J.; Crowley, Jane; Vimr, Eric; Silver, Richard P.
CORPORATE SOURCE: (1) Lab. Bacterial Polysaccharides, Cent. Biologics Res. Review, 8800 Rockville Pike, Bethesda, MD 20892 USA
SOURCE: Glycobiology, (1997) Vol. 7, No. 5, pp. 697-701. ISSN: 0959-6658.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Escherichia coli K1 produces a capsular polysaccharide of alpha(2-8) poly-N-acetylneuraminic acid. This polysaccharide is an essential virulence factor of these neuropathogenic bacteria. The genes necessary for the **synthesis** of neuNAc were localized to a plasmid containing the neuBAC genes of the K1 gene cluster. Cells harboring the neuB+ allele in an aldolase (nanA-) negative background produce neuNAc in vivo. Enzymatic **synthesis** of neuNAc could be demonstrated in extracts of cells harboring an expression plasmid (pNEUB) containing the neuB gene alone. NeuNAc **synthetase** was purified to homogeneity from extracts of cells harboring pNEUB. The molecular weight of the purified enzyme is 40 kDa, similar to that predicted by the nucleotide sequence of the neuB gene. The amino terminal sequence of the purified protein matches that predicted by the nucleotide sequence of the neuB gene. NeuNAc **synthetase** catalyzes the formation of neuNAc as indicated by its coupling to the CMP-neuNAc **synthetase** reaction. The enzyme condenses **manNAc** and PEP with the release of phosphate. The E. coli neuNAc **synthetase** is specific for **manNAc** and PEP, unlike rat liver enzyme that utilizes N-acetylmannosamine-6-phosphate to form neuNAc-9-PO-4. This represents the first report of a purification of a sialic acid **synthetase** from either a eukaryotic or prokaryotic source to homogeneity. These experiments clearly demonstrate an aldolase-independent sialic acid

L7 ANSWER 4 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:202703 BIOSIS
DOCUMENT NUMBER: PREV199799501906
TITLE: An efficient process for production of N-acetylneuraminic acid using N-acetylneuraminic acid **aldolase**.
AUTHOR(S): Mahmoudian, M. (1); Noble, D.; Drake, C. S.; Middleton, R. F.; Montgomery, D. S.; Piercey, J. E.; Ramlakhan, D.; Todd, M.; Dawson, M. J.
CORPORATE SOURCE: (1) Med. Res. Cent., Glaxo Wellcome Res. Dev., Gunnels Wood Rd., Stevenage, Hertfordshire SH1 2NY UK
SOURCE: Enzyme and Microbial Technology, (1997) Vol. 20, No. 5, pp. 393-400.
ISSN: 0141-0229.
DOCUMENT TYPE: Article
LANGUAGE: English

AB N-acetyl-D-neuraminic acid (Neu5Ac) **aldolase** (EC 4.1.3.3) has been reported for **synthesis** of Neu5Ac, but there are no reports of processes which do not have significant drawbacks for large-scale operation. Here, Neu5Ac **aldolase** from an overexpressing recombinant strain of Escherichia coli has been used to develop an immobilized enzyme process for production of Neu5Ac. The enzyme was immobilized onto Eupergit-C and could be reused many times in the reaction. Base-catalyzed epimerization of N-acetyl-D-glucosamine (GlcNAc) yielded GlcNAc/N-acetyl-D-mannosamine (**ManNAc**) mixtures (c 4:1) which could be used directly in the **aldolase** reaction; however, inhibition of the enzyme by GlcNAc limited the concentration of **ManNAc** which could be used in the reaction by this approach. This necessitated the addition of a large molar excess of pyruvate (five- to seven-fold) to drive the equilibrium over to Neu5Ac; nevertheless, a method has been developed to remove the excess pyruvate effectively by complexation with bisulfite, thus allowing Neu5Ac to be recovered by absorption onto an anion-exchange resin. In a second approach, a method has been developed to enrich GlcNAc/**ManNAc** mixtures for **ManNAc**. **ManNAc** can be used at high concentrations in the reaction, thus obviating the need to use a large molar excess of pyruvate.

Neu5Ac can be isolated from such reaction mixtures by a simple crystallization. This work shows the importance of integrated process solutions for the effective scale-up of biotransformation reactions.

L7 ANSWER 10 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1985:245274 BIOSIS
DOCUMENT NUMBER: BA79:25270
TITLE: PURIFICATION AND PROPERTIES OF N ACETYLNEURAMINATE LYASE EC-4.1.3.3 FROM ESCHERICHIA-COLI.
AUTHOR(S): UCHIDA Y; TSUKADA Y; SUGIMORI T
CORPORATE SOURCE: KYOTO RES. LAB., MARUKIN SHOYU CO., LTD., UJI, KYOTO 611.
SOURCE: J BIOCHEM (TOKYO), (1984) 96 (2), 507-522.
CODEN: JOBIAO. ISSN: 0021-924X.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB N-Acetylneuraminate lyase [**N-acetylneuraminic acid aldolase** EC 4.1.3.3] from E. coli was purified by protamine sulfate treatment, fractionation with (NH4)2 SO4, column chromatography on DEAE-Sephacel, gel filtration on Ultrogel AcA 44 and preparative polyacrylamide gel electrophoresis. The purified enzyme preparation was homogenous on analytical polyacrylamide gel electrophoresis, and was free from contaminating enzymes including NADH oxidase and NADH dehydrogenase. The enzyme catalyzed the cleavage of **N-acetylneuraminic acid** to **N-acetylmannosamine** and **pyruvate** in a reversible reaction. Both cleavage and **synthesis** of **N-acetylneuraminic acid** had the same pH optimum .apprx. 7.7. The enzyme was stable at pH 6.0-9.0, and was thermostable up to 60.degree. C. The thermal stability increased up to 75.degree. C in the presence of **pyruvate**. No metal ion was required for the enzyme activity, but heavy metal ions such as Ag+ and Hg2+ were potent inhibitors. Oxidizing agents such as N-bromosuccinimide, I, and H2O, and SH-inhibitors such as P-chloromercuribenzoic acid and HgCl2 were also potent inhibitors. The Km values for **N-acetylneuraminic acid** and N-glycolylneuraminic acid were 3.6 mM and 4.3 mM, respectively. **Pyruvate** inhibited the cleavage reaction competitively; K1 was calculated to be 1.0 mM. In the condensation reaction, N-acetylglucosamine, N-acetylgalactosamine, glucosamine and galactosamine could not replace **N-acetylmannosamine** as substrate, and phosphoenolpyruvate, lactate, .beta.-hydroxypyruvate and other **pyruvate** derivatives could not replace **pyruvate** as substrate. The MW of the native enzyme was estimated to be 98,000 by gel filtration methods. After denaturation in sodium dodecyl sulfate or in 6 m guanidine-HCl, the MW was reduced to 33,000, indicating the existence of 3 identical subunits. The enzyme could be used for the enzymatic determination of **sialic acid**; reaction conditions were devised for determining the bound form of **sialic acid** by coupling neuraminidase from Arthrobacter ureafaciens, lactate dehydrogenase and NADH.